（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K945-M

Specification：96T

Measuring instrument：Microplate reader (560-580 nm)

Detection range：1.446-500 μmol/L

Elabscience® Total Iron Binding Capacity (TIBC) And Serum Iron Assay Kit (Direct Method)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Intended use

This kit can be used to measure the total iron binding capacity (TIBC) and serum iron content in serum and plasma samples.

Detection principle

The amount of iron bound to transferrin in the blood is called serum iron. The maximum ability of transferrin to bind iron is called total iron binding capacity (TIBC), and iron saturation refers to the percentage of serum iron binding capacity to total iron binding capacity.

The detection principle of this kit: add an excess of iron to the sample and detect the total iron content. The content of unbound iron was detected when transferrin was saturated with iron. The total iron binding capacity of the sample is obtained by subtracting the content of unbound iron from the total iron content. The sample is not added with iron, and the total content of iron bound to transferrin and free iron is detected. Meanwhile, the content of free iron that has not bound to transferrin is detected. The serum iron content is obtained by subtracting the latter from the former.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	-20°C, 12 months
Reagent 2	Iron Stock Solution	1 mL × 1 vial	-20°C, 12 months
Reagent 3	Chromogenic Agent	2.8 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Iron Released Agent	2.4 mL × 1 vial	-20°C, 12 months
Reagent 5	10 mmol/L Iron Standard Solution	1 mL × 1 vial	-20°C, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:
Microplate reader (560-580 nm, optimum wavelength: 570 nm)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of iron working solution:
Before testing, please prepare sufficient iron working solution according to the test wells. For example, prepare 500 μL of chromogenic working solution (mix well 10 μL of iron stock solution and 490 μL of buffer solution). The iron working solution should be prepared on spot and used up within 8 h.

③ The preparation of 1 mmol/L standard solution:
Before testing, please prepare sufficient 1 mmol/L standard solution according to the test wells. For example, prepare 500 μL of 1 mmol/L standard solution (mix well 50 μL of 10 mmol/L iron standard solution and 450 μL of buffer solution). The iron working solution should be prepared on spot and used up within 8 h.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.05**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.5**
1 mmol/L standard (μL)	0	10	20	30	40	60	80	100
Buffer Solution (μL)	200	190	180	170	160	140	120	100

Sample preparation

① **Sample preparation**

Serum and plasma: Detect directly.

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Rat serum	1
Horse serum	1
Chicken serum	1
Rabbit serum	1
Mouse urine	1
Porcine serum	1
Fetal bovine serum	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

If test plasma samples, it is recommended to use heparin an anticoagulant. Ethylenediaminetetraacetate, sodium oxalate, sodium citrate or sodium citrate should not be used.

Operating steps

① Standard wells: Add 50 μL of standard solution with different concentration to the wells.
A wells (unbound iron), B wells (total iron binding capacity and unbound iron),
C wells (free iron), D wells (iron bound to transferrin and free iron): Add 50 μL of sample to the wells.

② Standard wells, C wells: Add 175 μL of buffer solution to the wells.
D wells: Add 125 μL of buffer solution to the wells.
A, B wells: Add 125 μL of iron working solution to the wells.

③ Mix fully, incubate at 37℃ for 10 min.

④ Add 25 μL of chromogenic agent to each well.

⑤ Mix fully, incubate at 37℃ for 10 min.

⑥ A wells: Add 50 μL of buffer solution to the wells.
B, D wells: Add 50 μL of iron released solution to the wells.

⑦ Mix fully, incubate at 37℃ for 10 min and measure the OD value at 570 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

$$\underset{(\mu mol/L)}{TIBC} = (OD_B - OD_A - b) \div a \times 1000^* \times f$$

$$\underset{(\mu mol/L)}{\text{Serum iron content}} = (OD_D - OD_C - b) \div a \times 1000^* \times f$$

$$\text{Iron saturation} = \frac{\text{Serum iron content}}{TIBC} \times 100\%$$

[Note]

1000*: 1 mmol/L = 1000 μmol/L

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	75	250	450
%CV	0.5	0.6	0.3

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	75	250	450
%CV	2.7	2.1	2.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	40	50	60
Observed Conc. (μmol/L)	41	49	60
Recovery rate(%)	103	98	100

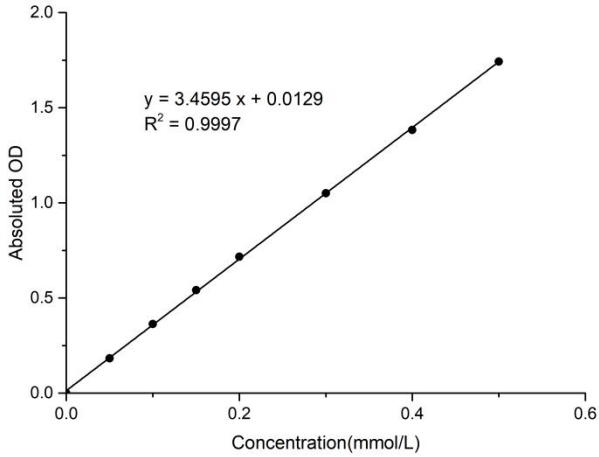
Sensitivity

The analytical sensitivity of the assay is 0.684 μmol/L TIBC. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	**0**	**0.05**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.5**
OD	0.050	0.231	0.411	0.586	0.759	1.091	1.428	1.787
	0.045	0.230	0.409	0.591	0.769	1.106	1.433	1.793
Average OD	0.048	0.231	0.410	0.589	0.764	1.099	1.431	1.790
Absoluted OD	0.000	0.183	0.363	0.541	0.717	1.051	1.383	1.743



Appendix Π Example Analysis

Example analysis：

Take 50 μL of human serum and carry the assay according to the operation steps. The results are as follows:

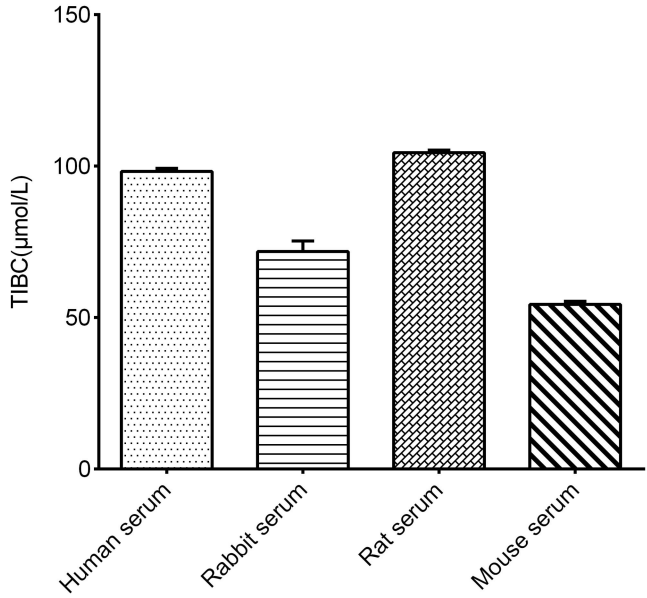
Standard curve: y = 3.4595 x + 0.0129, the OD value of A well is 0.944, the OD value of B well is 1.296, the OD value of C well is 0.113, the OD value of D well is 0.237 and the calculation result is:

TIBC (μmol/L) = (1.296 - 0.944 - 0.0129) ÷ 3.4595 × 1000 = 98.02 μmol/L

Serum iron content (μmol/L) = (0.237 - 0.113 - 0.0129) ÷ 3.4595 × 1000 = 32.11 μmol/L

Iron saturation = 32.11 ÷ 98.02 × 100% = 33%

Detect human serum, rabbit serum, rat serum and mouse serum according to the protocol, the result of TIBC is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.